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Cell-contact and -architecture of malignant cells and their relationship to metastasis

Avraham Raz[1] & Avri Ben-Ze'ev[2]
Departments of [1] Cell Biology and [2] Genetics, Weizmann Institute of Science, Rehovot 76100, Israel ([1] address for offprints)

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Summary

The interaction of metastatic cells with the host environment occurs, to a large extent, through the cell surface, and the cell cytoskeletal system controls the distribution and motility of cell surface receptors. During metastasis, tumor cells migrate from one organ to another, and the dynamic properties and mechanochemical deformability of disseminated cells play a central role in the process. The studies described hereunder suggest an interrelationship between the cytoskeleton and cell adhesion, which can control and uagment the expression of the metastatic phenotype of neoplastic cells.

Introduction

The molecular mechanisms underlying the transformation of normal cells to cancer cells constitute the main focus of interest in cancer research in the last few decades. The malignant properties of cancer are manifested in the propensity of the cells to spread from the primary tumor to distant organs where they can grow and produce metastases, the major cause of death from cancer. New approaches to the treatment of metastasis will be forthcoming only when the pathobiology of this lethal process is understood more clearly. For this reason one of the primary goals of cancer research today should be the elucidation of tumor cell dissemination and the growth of cancer metastases. The process of metastasis consists of several sequential steps which include detachment of tumor cells from the primary tumor mass, invasion into the circulation, arrest in the capillary bed, extravasion, proliferation in organ parenchyma, and the interaction of tumor cells with the host immune system throughout the entire process.

After more than a decade of intensive studies, it is now well accepted that tumors are heterogeneous and that only minor subpopulations of cells in malignant rodent and human neoplasms are capable of completing all the steps required to produce fatal metastases. Indeed, many clinical observations support the experimental data that metastasis is not due to the random survival of tumor cells. The patterns of metastasis of different tumors are predictable, and cells from a metastasis frequently metastasize to other organs to form secondary or tertiary metastases (for review, see Ref. 1–4). In vivo and in vitro selection techniques have yielded paired metastatic and nonmetastatic cell lines and clones. The availability of cell variants with different metastatic properties affords the opportunity to determine the various cellular and molecular phenotypes of metastatic cells.

It is probable that the factors which control metastasis vary from one tumor system to another, and even among different target organs in the same host. Despite these obvious variabilities, common denominator processes (minor or major) for metastasis must exist. Based on this reasoning, many experimental attempts have been made to correl-

ate different properties of malignant cells with their metastatic capabilities. Since the process of metastasis involves a dramatic alteration in cell morphology (Fig. 1), the question arises of the possible interrelation between cell shape-responsive metabolic and functional controls and the metastatic capability of malignant cells.

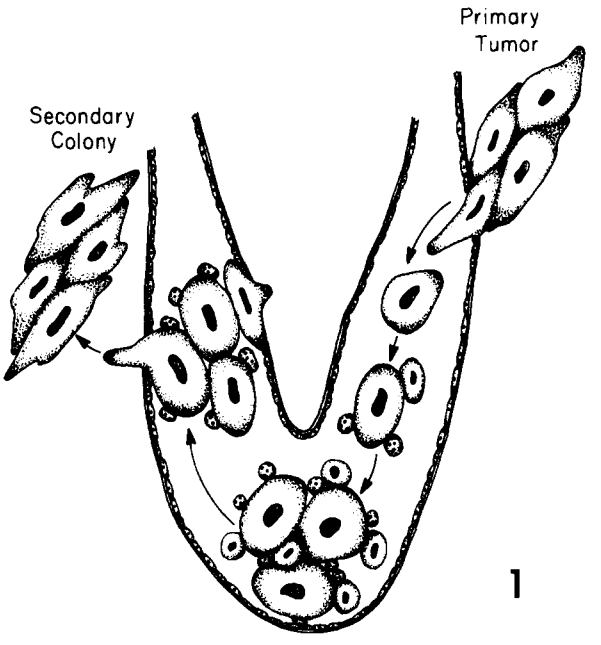

Fig. 1. Schematic representation of the process of metastasis via the circulation. The metastatic cells penetrate into the circulation via capillaries that reach the primary tumor. Tumor cell emboli are formed in the capillary after interaction of the metastatic cells with the immune system (small cells) and this causes the arrest of the circulating metastatic cells, followed by extravasation and the formation of secondary metastatic colonies.

Cell locomotion and metastasis

The release of tumor cells from the primary site of growth depends on the capability of tumor cells to break existing contacts with neighboring cells or with the connective tissue.

The translocation of metastasizing tumor cells from the primary site of growth to the development of secondary tumor foci at the target organ necessitates both active locomotion and directionality. The force-generating system of migratory cells should be produced from cellular structures such as the leading lamella, the posterior trailing edge, ruffles, the cytoskeletal-contractile elements, focal contacts and the cellular energy balance. The polarity of the cell which controls directional locomotion is most probably governed by the above intracellular parameters and external guiding stimuli like chemotactic gradients, the presence of unique adhesive-promoting constituents of the extracellular matrix, the organ microenvironment, etc.

Tumor invasion is frequently seen as a direct extension of cell penetration into the host parenchyma. Strauli and Weiss [5] suggest that movement might be mediated by motility of individual cells or groups of cells at the advancing edge of tumor protrusions. The dissemination of metastatic basal cell carcinomas, the satellites, or in transit metastases of melanoma, and the spread of many carcinomas and sarcomas can be best explained by embolic spread through interstitial compartments [6]. The tumor cells may then penetrate as either single cells or small cell clumps into blood vessels or the lymphatic system, where they may be transported to distant sites. The arrest of these cells in organs may be influenced by the formation of tumor emboli as a result of self-aggregation and/or interaction with circulating host cells [7–11]. The site of arrest and subsequent proliferation of the tumor cells may be either specific or nonspecific. The notion of site-specific metastatic spread stems from observations of a nonrandom distribution pattern of metastases [12–14] and from the presence of tumor cell variants exhibiting preferential colonization in specific organs [15–19]. Specific tumor cell arrest presumably depends on the interactions of tumor cells with the capillary endothelium or with exposed basement membranes and intercellular matrices [20–22] and the attainment of an extravascular position is believed to involve an active locomotion similar to that responsible for the initial invasion into the blood vessels.

The involvement of cell locomotion throughout the metastatic spread raises the question of the possible relationships between the cellular manifestation of the metastatic capabilities in vivo and cell motility as assayed in vitro. The system which enabled us to approach this question consists of

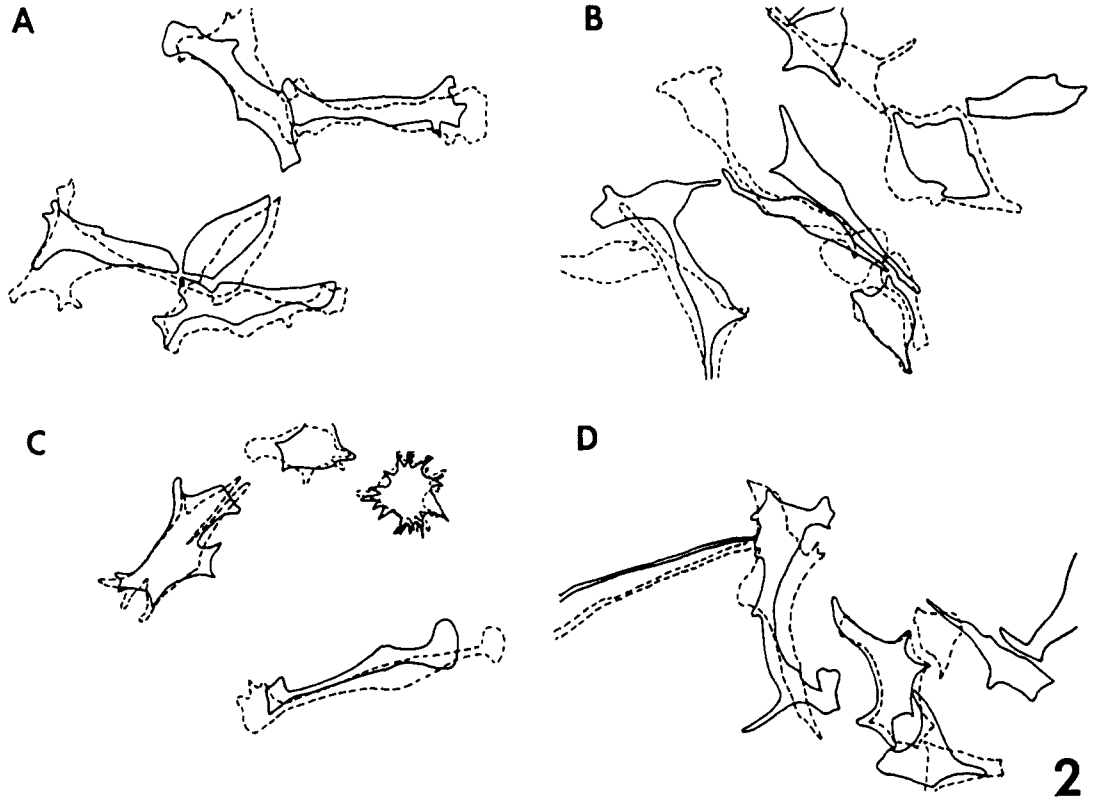

Fig. 2. The locomotion of high and low metastatic cells. The locomotion of two low metastatic (B16-F10^{Lr-6} (A), UV-2237-15 (C)) and two high metastatic (B16-F10 (B) and UV-2237-IP3 (D)) cell variants of the B16 melanoma and UV-induced fibrosarcoma was analyzed. Cells were plated on glass coverslips and the microscopic image of a few cells was traced on a semitransparent paper 24 h (solid lines) and 40 h (discontinuous lines) after plating.

pairs of closely related cell lines and clones originally derived from the same parental tumor, i.e. B16 melanoma, K1735 melanoma, UV-2237 fibrosarcoma, and BALB/c-A31 angiosarcoma [23–26]. Initially, monolayers of low and high metastatic B16 melanoma cell lines (B16-F10^{Lr-6} and B16-F10) and of the UV-2237 fibrosarcoma (clone 15 and IP3) were cultivated at 37° C while viewed with an inverted microscope equipped with a video camera, and the cells were photographed at various time intervals. As shown in Fig. 2, the high metastatic cells of both tumor systems show considerably higher locomotory activity as compared with their low metastatic counterparts. These results are reconfirmed by the significantly higher level of chemokinetic motility of B16-L4 (high metastatic) in comparison with the B16-F1 parental cells [27]. These differences are further amplified when the cells are seeded on a carpet made of the extracellular matrix component fibronectin, which has been shown to mediate haptotactic movements of B16 melanoma cells [28]. To quantitate the locomotory activity of low and high metastatic cells, cells of a recently derived tumor system, K1735 melanoma, are plated on colloidal gold-coated glass coverslips (prepared according to Albrecht-Buehler [29]). From the phagokinetic tracks formed by individual cells it becomes apparent that the low-metastatic cells are largely immobile and do not move over larger distances, while the high metastatic cells show considerably higher locomotory activity with a mean rate of movement of $5.0 \pm 2.2\,\mu$m/hr (Fig. 3) [24]. Migration analysis of Lewis lung carcinoma cells [30] and of rat mammary adenocarcinoma cells [31] out of glass capillary tubes also show a direct relationship between the metastatic

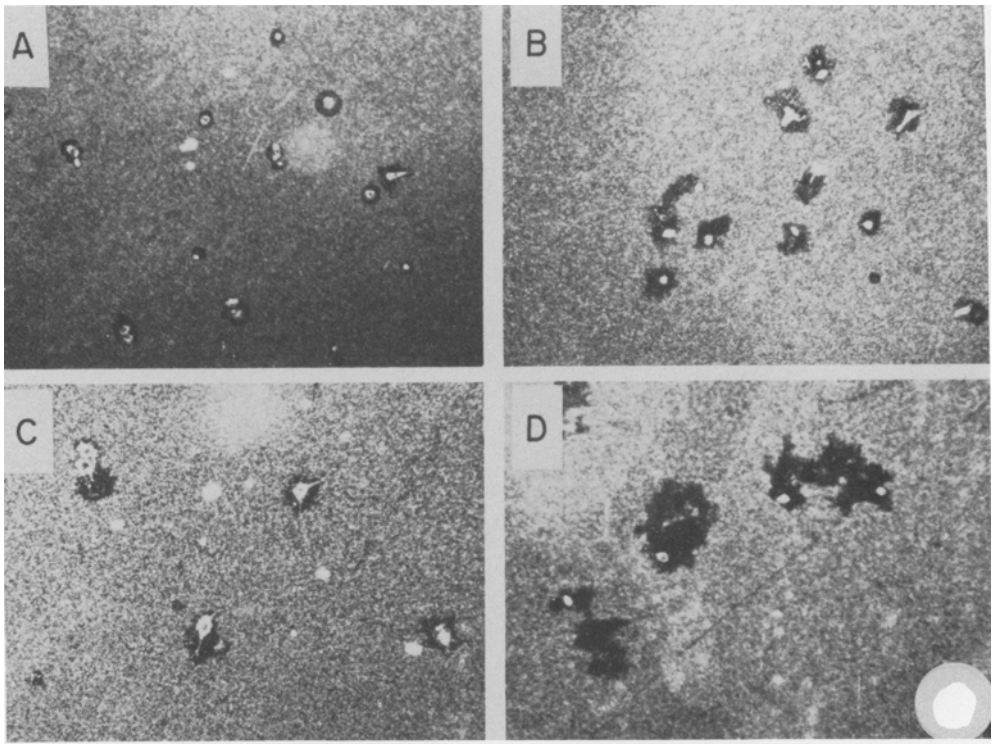

Fig. 3. Phagokinetic tracks of high and low metastatic cell variants. K-1735 melanoma variants displaying low (A, C) and high (B, D) metastatic potential were seeded on substrates with colloidal gold and their phagokinetic tracks were visualized by dark-field illumination after 1 day (A, B) and 3 days (C, D). (Reprinted with permission from Volk *et al* [24]).

capability and the capacity of cells to migrate.

Cell-shape, cell-adhesion and their relationship to metastasis

When attached and spread on a solid support, normal cells grow until the surface is completely covered by a crowded sheet (monolayer) of cells which then stop proliferating, a phenomenon known as anchorage dependence [32]. The proliferation of untransformed cells will occur when hormonal growth factors are available, but these growth factors are not effective if the cells are placed in suspension culture [33]. Thus, anchorage dependent growth is most probably cell-contact and -shape dependent growth, since the addition of small glass fibrils to suspended growth-arrested cells (in a semisolid medium) enables cell proliferation after the cells have attached to the fibrils [34]. Furthermore, in an elegant study, Folkman and Moscona [35] show that by accurately controlling the amount of cell spreading on the substrate (by modulating cell morphology between flat and spherical), that a small change in cell spreading has a large effect on the extent of DNA synthesis. The loss of anchorage-dependent growth control is one of the major manifestations of tumorigenicity [36]. Since not all tumor cells in the primary neoplasm progress towards malignancy at the same rate, primary tumors are populated by cells of different malignant potential that can be selected to obtain variant cloned cell lines [1–3].

Thus, the question arises as to the possible relationship among cell shape, cell spreading, cytoskeleton organization and the metastatic phenotype of tumor cell variants which are characterized in vivo. First, we studied the metastatic phenotype of neoplastic cells, since Folkman and Greenspan [37] postulate that the loss of growth regulation by cell

shape may be a central feature of cell malignancy. This hypothesis is substantiated by the report that the tumorigenic phenotype of x-irradiated $10T^{1}/_{2}$ cells is expressed only in dense cell cultures or in cells cultured on nonadhesive substrates, where the cells have a round configuration. It was therefore suggested that the transformed phenotype of these cells may be influenced by changes in cell shape [38]. More recently, Wittelsberger et al. [39] in an analysis of mouse fibroblast variants expressing a continuum of decreasing regulation of growth in vitro suggest that the progressive loss of cell-shape-reponsible metabolic controls may be related to the phenomenon of tumor progression. We confirm the above results by an analysis of fibroblastic cell lines of the 3T3 series which finds that the growth of non-neoplastic cells in vitro is highly sensitive to alterations in cell configuration, while the growth regulation of transformed cells is partially or fully uncoupled from cell spreading on the substrate [40]. In attempting to mimic the morphological changes imposed on cells that grow in contact with the tissue at the primary tumor site and their subsequent movement into the circulation as suspended cells, similar morphological changes in cell shape are induced by controlling the extent of cell spreading on the substrate, using the poly(HEMA) technique [35, 41]. When analyzing the rate of DNA synthesis as a function of cell spreading on the substrate in cell variants of the B16 melanoma and UV-2237 fibrosarcoma tumor systems displaying low, intermediate and high metastatic potential, we find a lack of direct correlation between the metastatic phenotype and the changes in DNA synthesis in response to changes in cell shape [40]. Nevertheless, the proliferation of the various metastatic cells is clearly affected by changes in cell configuration to various extents in all cell lines tested [40]. Next, we ask whether proliferation of cells under conditions of controlled cell shape may alter the metastatic phenotype. B16-F1 melanoma K1735-11 melanoma and UV-2237-15 fibrosarcoma cells (Fig. 4) are plated on substrates coated with varying concentrations of poly(HEMA) which allow the control of cell shape between flat to spherical. Growth under these conditions is used as a model system to mimic the different growth patterns obtained in vivo in the extravascular and intravascular systems. We find that the growth of these various tumorigenic cells in vitro in a spherical configuration induces a marked increase in their metastatic cability in vivo (Fig. 4). In addition, we observe a much faster attachment and spreading on the substrate of cells replated in vitro following suspension culture as compared to cells grown as a monolayer. The alterations in the metastatic capabilities of the tumor cells as shown in Fig. 4 are reversible upon reattachment and spreading of cells on the substrate for 24–48 h [42], suggesting a central role for changes in cell shape in the modulation of metastatic capability. Metastases in vivo do not have to be caused by cells with the highest metastatic potential, but by cells endowed with the metastatic phenotype [43]. In addition, signals from the microenvironment can regulate the expression of various genetic programs, which in turn may activate or repress various cellular activities that may affect the metastatic potential [44]. The reversible modulation of the metastatic capability by changes in cell shape is another example which is compatible with these suggestions. More recently, Stackpole *et al.* [45] report that the B16 LM3/G3.26 cloned cell line loses its in vivo colonizing activity during extended propagation in culture. When these long-term-cultured cells are cultivated for 72 hr as loose unattached aggregates, strong colonizing activity, as well as other biological properties typical of the original clone are restored [45]. Thus, the 'loss' of colonizing activity by G3.26 cells during prolonged monolayer culturing, similar to the low colonizing potential of B16-F1 monolayer cells [42], does not result from the loss of the inherent colonizing potential, but rather because of 'masking' of an otherwise stable phenotype during cell adaptation to continuous culturing in the form of a monolayer.

Metastatic capacity, cellular configuration and adhesive properties of neoplastic cells have been modulated by drugs and growth factors. Treatment of low-metastatic Lewis lung carcinoma cells (P-29) with dimethylsulfoxide (DMSO) resulted in enhanced lung-colonizing ability and in an increase in their homotypic aggregation and adhesion to culture dishes [46]. The effects induced by DMSO

are very similar to those induced by the growing of B16 melanoma cells in a spherical configuration [42]. Moreover, the elevated metastatic phenotypes induced by DMSO in P29 cells and by spherical configuration in B16 melanoma cells is reversible [46, 42]. In a different study, human breast cancer cells (T-47D) treated with either prolactin, steroids or growth hormone show alterations in cell shape, adhesion and lipid accumulation [47]. These results, when compared with pathological observations, lead the investigators to suggest that prolactin which induces cell rounding, reduced cellular adhesion and an increased content of intracytoplasmic lipid droplets in T-47D breast cancer cells, may play a role in determining the invasive and metastatic potential of human breast cancer cells [47]. In contrast, treatment of B16-F1 cells with a tertiary amine, local anesthetics, results in cell rounding, loss of microvilli and reduced ability to form colonies of tumor cells in the lung [48]. Such drugs also cause decreased but reversible cell-adhesion in both homotypic and heterotypic cell adhesion assays. These are opposite effects to those induced by suspension growth conditions [42].

The studies summarized above raise the question of whether there are spontaneous tumor systems which display similar variations in cell configuration and metastatic phenotype, and whether in such systems these properties are similar to those obtained during the experimental modulations of morphological and metastatic properties. When substrate-adherent variant cells are selected from a tumorigenic suspension growing S49 mouse lymphoma cell line, it is found that the adherent variant is less tumorigenic than the parental cell line [49]. Another study shows that the selection of cells that are adherent to plastic from a suspension culture of a highly malignant T-cell lymphoma is followed by the loss of the metastatic capability in the adherent cell variant [50]. Similarly, cloned Lewis lung metastatic carcinoma cells isolated from metastatic lung nodules exhibit enhanced dissemination capacity in vivo and have a round cell morphology in vitro as compared to the parental cell line [30].

The recently established tumor cell variants of a spontaneous adenocarcinoma of the pancreas in BDX rats (BSp73) are of special interest to us, because these variants display in a stable fashion several characteristics which we obtained in the B16 melanoma system under culture conditions that modulated cell shape. By a subcutaneous transplantation of the BSp73 nonmetastasizing (AS) and metastasizing (ASML) cells, variants are obtained which maintain a stable phenotype after in vitro recloning [51]. The nonmetastasizing variant cells attach and spread on the culture dish, whereas the ASML cells which spontaneously metastasize to the lungs via the lymphatic vessels adhere and grow as spherical cells but fail to spread on the culture dish [51].

We have recently reported that the metastasizing ASML variant cells adhere to the substrate via thick cytoplasmic protrusions, but are removed from the substrate by trypsin EDTA more slowly than the nonmetastasizing AS variant cells [53]. The spheroid configuration of the metastasizing ASML cells is found to be a primary manifestation of cellular properties, since these cells can not be induced to spread when plated on an adhesion-promoting substrate, such as the extracellular matrix derived from bovine cornea endothelial cells, or on a concanavalin A-coated substrate, or by the addition of dibutiryl cAMP to the medium [53]. The BSp73 system thus brings to the extreme our previous observations on the linkage between the degree of cell spreading on the substrate and the metastatic phenotype of the cells [23–25, 53].

The actin-cytoskeleton of metastatic cells

Cell shape in vivo is determined by the interaction of the cell with the extracellular matrix and/or with neighbouring cells [54]. These points of cellular interaction with the environment are characterized by the specific organization of cytoskeletal elements in characteristic molecularly defined structures. Specific integral membrane proteins mediate these interactions between the cytoskeleton and the extracellular matrix and with the neighbouring cells and these interactions have a major role in determining pattern formation and tissue morphogenesis [54, 55].

Growth-related cellular functions are transmitted from the cell surface through an organized cytoskeleton, since interference with the microtubule or microfilament organization may dramatically affect the initiation of DNA synthesis [56, 57]. Furthermore, various cytoskeletal elements very often undergo rapid transient changes in their organization in response to growth factors [58, 59].

Tumor cells, like many nontumorigenic cells, possess well developed cytoskeletal networks [23, 36, 60]. Active directional motility and the capability of metastasizing cells to deform may also depend on cytoskeletal organization. Information on the possible role of cytoskeletal elements in metastasis was first acquired from studies utilizing drugs which disrupt the cytoskeleton. When TA3 ascites cells are treated in culture with cytochalasin B (CB), which disrupts microfilament organization, and the cells subsequently injected into syngeneic hosts, a marked alteration in the pattern of metastasis is noted [61]; an increased number of extrapulmonary metastatic foci are detected, and it is suggested that this increase results from changes in cell configuration induced by the disruption of the microfilament system. The disruption, in turn, enables the cells to traverse the lung capillary bed and to lodge in other organs [61]. B16-F10 cells treated in culture with CB, colchicine (to depolymerize microtubules), or with both drugs together exhibit a marked reduction in the formation of lung metastases when compared to control untreated cells [62]. By monitoring the distribution, survival, and growth of intravenously injected cells we know that the altered growth patterns are not a consequence of differences in the initial organ distribution of the injected tumor cells, which were similar for all the groups. However, the disruption of these cytoskeletal filamentous systems in B16-F10 cells results in a reduced number of cells retained within the lung 1 hr post injection, and in an increase in the number of cells found in extrapulmonary organs. The subsequent relocation of these cells implies that cytoskeletal elements of malignant cells may be important in determining the organ distribution of metastasis. Similarly, the treatment of B16 melanoma cells with tetracaine results in a reversible disruption of actin-containing microfilaments, and in a reduced capacity to colonize the lung [48].

The studies described above prompted us to analyze the organization of the major cytoskeletal filamentous networks in three tumor systems derived from the mouse; the UV-2237 fibrosarcoma, the K1735 melanoma and the BALB/c angiosarcoma. Significant differences between low and high metastatic cell variants in these tumor cell systems are detected predominantly in the actin-containing network. The high-metastatic variants exhibit distorted actin bundles [23, 26]. Randomly cloned cell lines from the parental K1735 melanoma are analyzed for the degree of actin organization and the in vivo lung colonization capability independently assayed. In nearly all cloned K1735 melanoma cell variants (14 out of 15) a correlation is found between the patterns of actin distribution and the metastatic potential. The low metastatic clones have an elaborate stress fiber system, while the organization of actin in the high-metastatic cells is mostly diffuse (Fig. 5). Furthermore, the organization of actin in these cells is a primary manifestation of a stable cellular phenotype, since it could not be modulated by varying the substrate. Plating of high-metastatic cells on a fibronectin-coated substrate, or on extracellular matrix produced by either human foreskin fibroblasts or by a low-metastatic cloned cell line, does not alter the cytoarchitecture of the cells to a significant degree (Fig. 6).

Nuclear magnetic resonance studies of low and high metastatic cell variants of the Dunning R337 rat prostatic adenocarcinoma reveal that the highly metastatic cell line has significantly longer water proton T1 relaxation times than the cells with low metastatic potential [63]. These differences in T1 may reflect the observed alterations in the organization of actin filaments, and the organization of actin filaments correlates with the metastatic potential of the various cell lines [63].

In human colonic epithelial cells, it was reported that the loss of actin organization appears to mark the transition of noninvasive benign colonic tumors to invasive malignant tumors [64]. This phenotypic transition may occur if an oncogene product acts at a late stage in tumor development on the cyto-

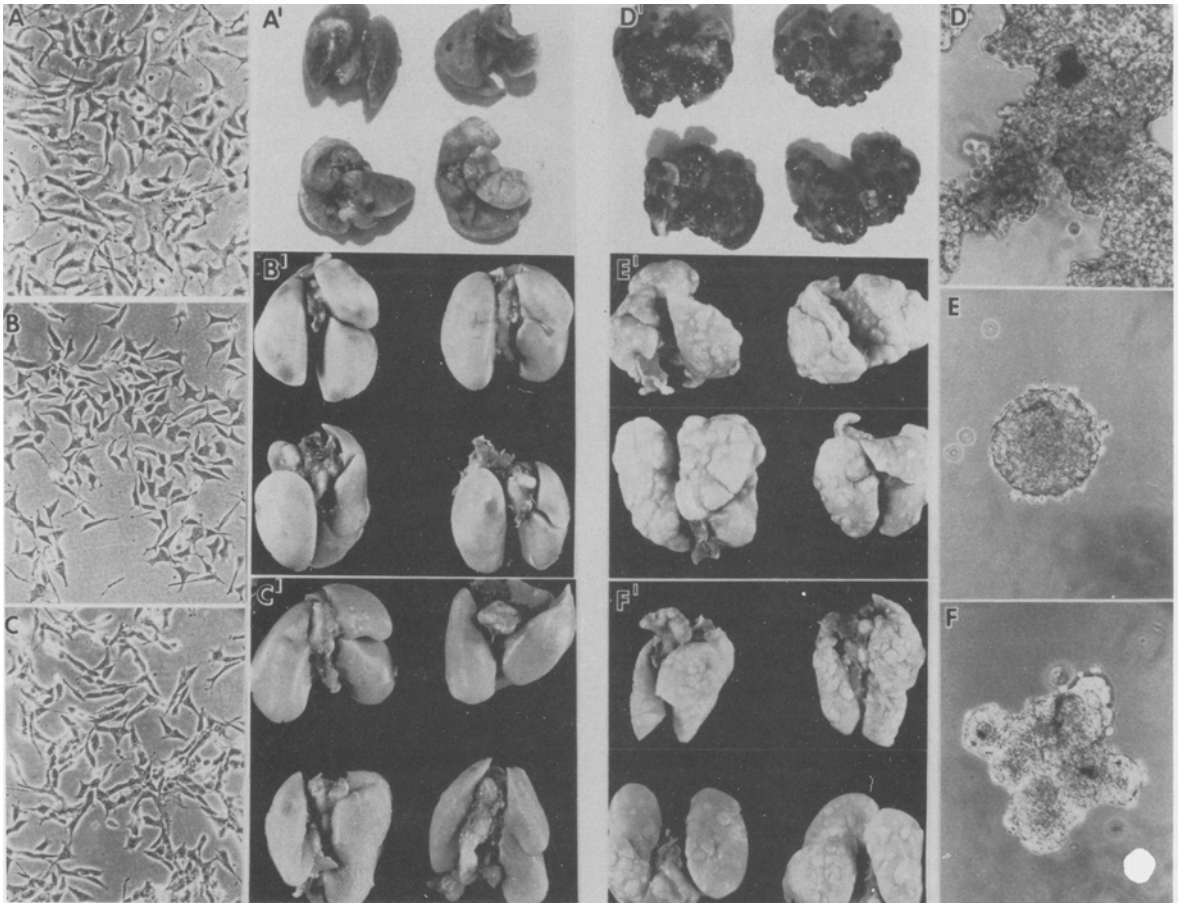

Fig. 4. The morphology and metastatic capability of malignant cell cultures grown in monolayer or in suspension cultures. B16-F1 melanoma (A, A', D, D') K-1735-11 melanoma (B, B', E, E') and UV-2237-15 fibrosarcoma (C, C', F, F') were grown in either monolayer (A-C) or for 3 days in suspension on poly(HEMA)-coated plates (D-F), and equal numbers of cells from single cell suspensions prepared from either the monolayer (A'-C') and the suspension cultures (D'-F') were injected into the tail vein of mice to assess their capability to form tumors in the lung.

skeleton, which could be one of its sites of action [64].

Could these differences in the organization of actin filaments between high and low metastatic cells also reflect differences in the mode of cell-substrate interaction in culture and cell-cell interaction in vivo? A major constituent of such interactions is vinculin, which is involved in the linkage of actin filaments to the cell substrate contacts (focal contacts) and cell-cell-contacts of the adherens type [65, 66]. Low-metastatic variants of UV-2237 fibrosarcoma and K1735 melanoma cells display relatively large vinculin-containing focal adhesion plaques (Fig. 7). In contrast, high metastatic variants of these tumors are loosely attached to the substrate, with fewer and smaller focal contacts [23, 24]. In agreement with these results, we find that in the BSp73 adenocarcinoma tumor system the low metastatic cells are flat on the substrate and display well developed vinculin-containing focal contacts, while the spherical and poorly adherent metastatic counterpart cells synthesize very little vinculin and have few, if any, vinculin plaques (see Fig. 10E and F). Furthermore, in a B16 melanoma cell variant, (B16-F1-NA) selected from the parental cell population for its reduced capability to form homotypic aggregation and which exhibits a markedly reduced propensity to form experimental

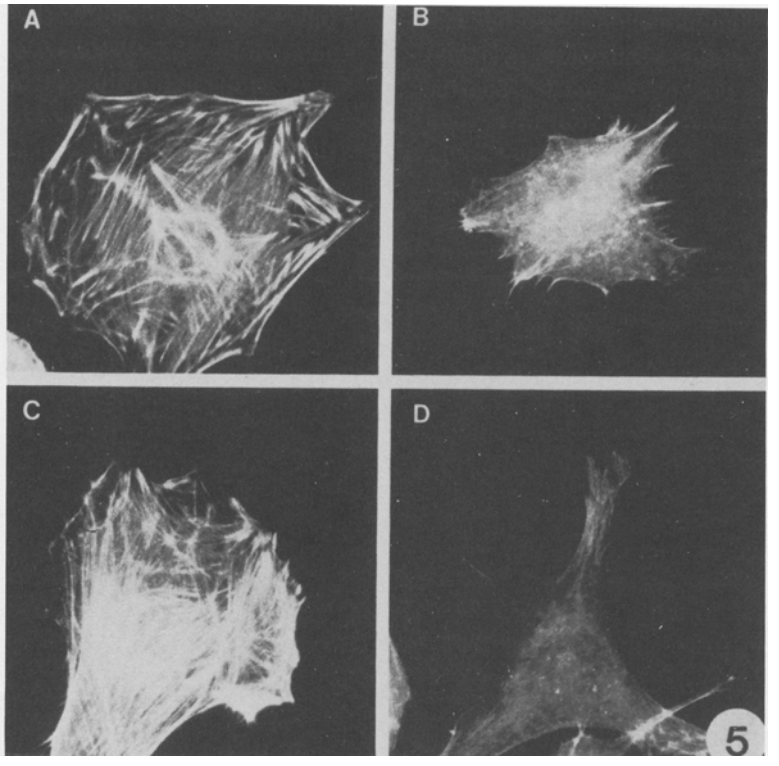

Fig. 5. The organisation of the actin-cytoskeleton in metastatic cell variants seeded on various substrata. Low metastatic (A, C) and high metastatic (B, D) cell variants of the K-1735 melanoma were seeded on either glass coverslips (A, B) or on coverslips coated with fibronectin (C, D). After 24 h the actin-cytoskeleton was visualized in fixed-permeabilized cells with rhodamine-phalloidin. Note that fibronectin coated substrata did not induce the formation of elaborate actin networks in high metastatic cells (D). (Reprinted with permission from Volk et al [24]).

metastases, the interaction of the cells with the substrate is mediated via numerous focal contacts, while the parental B16-F1 cells exhibit, in comparison, a smaller number of focal contacts [53].

Electron microscopical analyses of local tumors that develop following subcutaneous inoculation of high and low metastatic K1735 melanoma cell variants reveal that low metastatic cells form compact tumors with numerous intercellular contacts of the adherens type, while the high metastatic cells form loose tumor masses with very few interconnections between the cells [25], in agreement with the way these cells interact with the substrate *in vitro* [23].

In a different approach, utilizing the latex particle adherence assay, it was found that a high malignancy of fibrosarcoma is regularly associated with a low cell surface adhesiveness, while a low malignancy line of the same type of tumor shows a high level of cell surface adhesiveness to the latex beads [67].

Finally, in view of the observation that the adhesion-promoting molecules tend to concentrate at the ventral (attached) face of the cells, which is in contact with the substrate [65, 66], we analyzed the adhesive properties of the apical (non-attached) face of the cells [68]. An instrument and a method were devised to measure the interfacial energy of interaction between inert substrates and cell monolayers. The values obtained by such measurements indicate that the high-metastatic cell variants of UV-2237 fibroblasts and K1735 melanoma tumor systems have a two-fold higher interfacial energy than their low metastatic cell counterparts [68]. Thus, the cells appear to have unevenly distributed adhesive molecules between the ventral and apical surfaces. Low metastatic cell variants interact

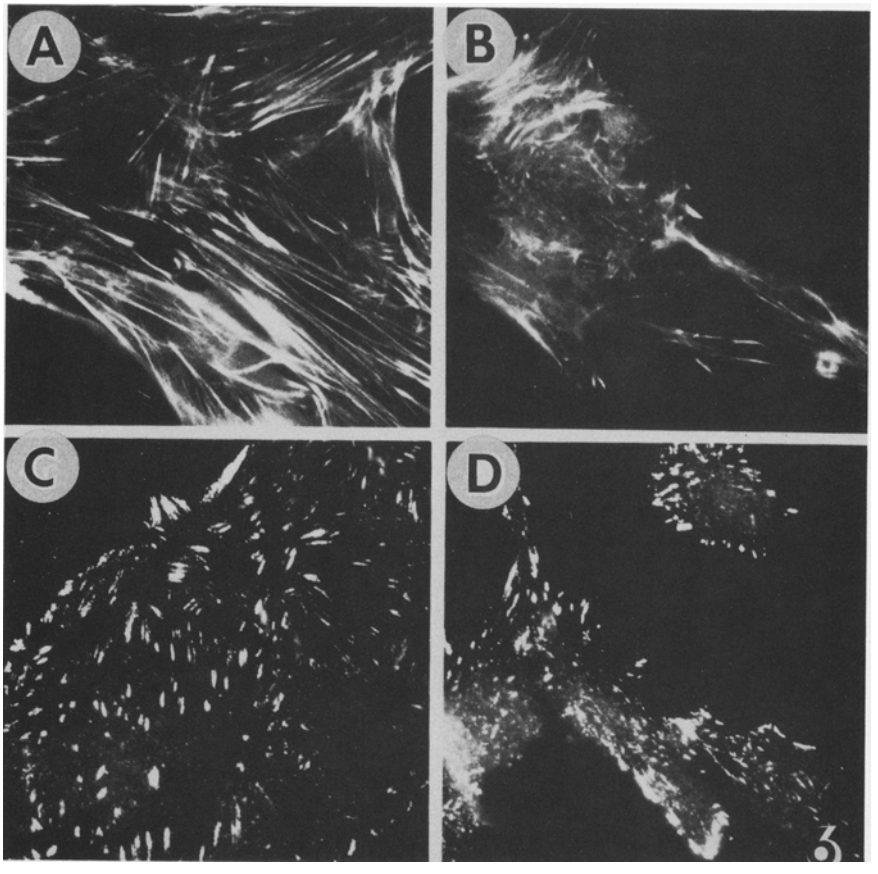

Fig. 6. The organization of actin filaments and vimentin containing focal contacts in high and low metastatic cell variants. Low metastatic (A, C) and high metastatic (B, D) variants of the UV-2237 fibrosarcoma were seeded on glass coverslips and the organization of actin (A, B) and vinculin (C, D) was analyzed using rhodamine-phalloidin for actin and a monoclonal antivinculin antibody. (Reprinted with permission from Raz and Geiger [23]).

strongly with the solid substrate via the ventral surface and poorly via the apical surface, while high metastatic cell variants display an opposite pattern of interaction with substrates.

Intermediate filaments (IF) and the metastatic phenotype

Intermediate filaments form a class of highly polymorphic and insoluble filaments that are divided into five groups (for recent reviews on intermediate filaments see 69). Cells of mesenchymal origin have IF composed of vimentin, muscle cells contain IF composed of desmin, neurofilaments contain three polypeptides, glial filaments are characterized by the fibrilar acidic protein and epithelial filaments contain cytokeratins (about 20–30 different proteins and desmosomes). The expression of IF is cell-type specific and relates to the embryonic pathways of differentiation. The practical use of IF biology for diagnostic purposes became apparent after it was found that, in general, the filaments maintain their ultrastructural and immunological properties during malignant transformation in vivo [70]. Carcinomas express keratins, muscle sarcomas desmin, nonmuscle sarcomas, lymphomas and leukemias express vimentin, while gliomas express the glial fibrillary acidic protein. Tumors originating from the sympathetic nervous system express neurofilaments. This can provide valuable information for diagnostic purposes in the

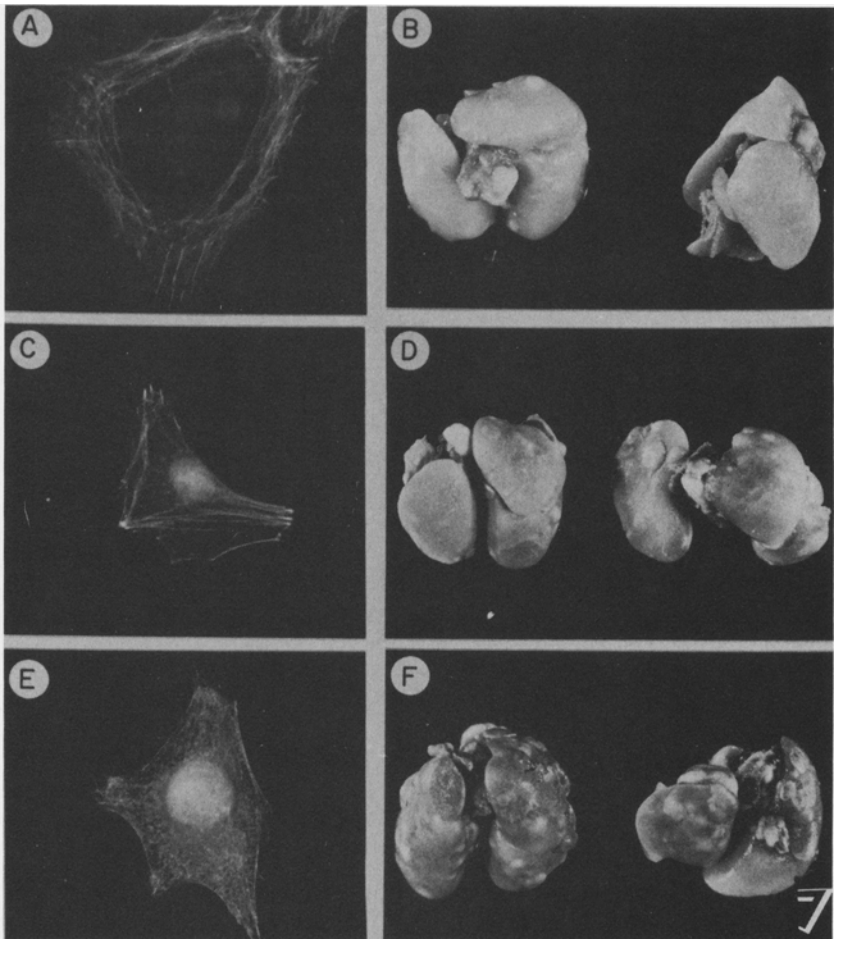

Fig. 7. The organization of the actin-cytoskeleton and the metastatic capability of randomly selected metastatic cell clones. Randomly selected clones of the K-1735 melanoma were analyzed for the organization of their actin cytoskeleton using rhodamine-phalloidin (A, C, E) and for their capacity to form metastatic colonies in the lung (B, D, F). (Reprinted with permission from Volk et al. [24]).

classification of tumors [71]. Metastases of solid tumors appear to retain the IF pattern characteristic of the primary and parent cell. However, several recent studies have demonstrated modulations and shifts in the expression of IF proteins in both normal and tumor cells and their metastases.

In our studies of cell-cell and cell-substrate contact-dependent regulation of cytoskeletal protein synthesis, we find that the transition from a well spread and flat morphology to a more rounded configuration, by altering the adhesive properties of the substrate, is accompanied by a dramatic repression in the synthesis of vimentin (the mesenchymal type IF protein) (Fig. 8, [72]). This reversible down regulation is characteristic of both normal and malignant metastatic cells [72]. The regulation of vimentin expression in relation to cell shape changes is not uncoupled in malignant cells. Nevertheless, it is interesting to note that a short period of cell culturing in a spherical configuration causes a dramatic decrease in vimentin synthesis, but also induces a marked increase in the malignant metastatic property of B16 melanoma cells [42]. These findings are similar to results obtained with small cell carcinoma of the lung, which has a high metastatic capacity, grows in suspension, but does

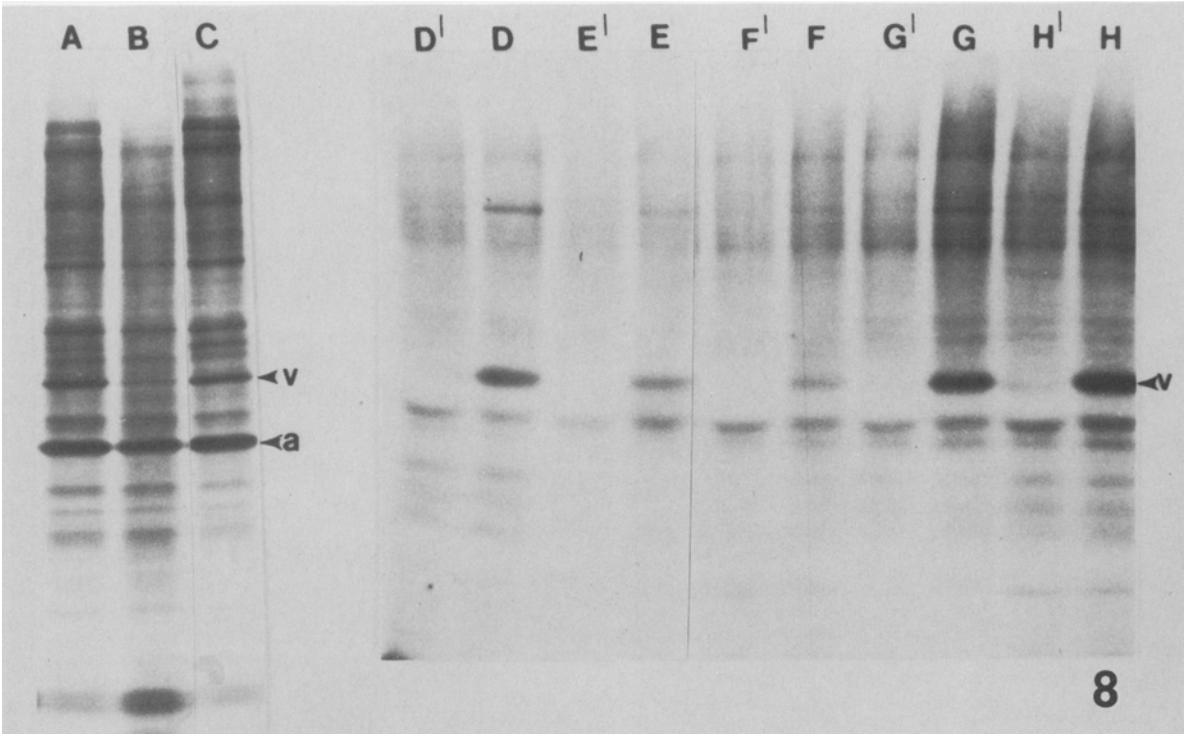

Fig. 8. Modulation of vimentin synthesis in B16 melanoma by changes in cell shape. B16-F1 melanoma cells were grown as a monolayer (A) or in suspension for 3 days on poly(HEMA)-coated plates (B), or were replated for 6 hr on plastic after 3 days suspension culture (C). The cells were pulse labeled with [^{35}S] methionine and equal amounts of radioactive proteins were analyzed by polyacrylamide SDS gel electrophoresis. D-H, immunoprecipitation with antivimentin antiserum or non-immune serum (D′-H′) from monolayer (D, D′), 1 day (E, E′), or 3-day (F, F′) suspension cultures and 6 hr (G, G′) and 24 hr (H, H′) replated cultures. a, actin; v, vimentin.

not express vimentin [73]. A study in which the organization of the vimentin network is disrupted by treating the cells with cycloheximide suggests that changes in the organization and expression of vimentin are related to the expression of the malignant metastatic phenotype [74]. Cycloheximide, a protein synthesis inhibitor, affects the vimentin network, but not the microtubules or the microfilaments, and causes a dramatic inhibition in vimentin synthesis, a concomitant reduction in vimentin mRNA content (Fig. 9), and alters the metastatic properties of B16 melanoma cells. The effects on both vimentin organization and expression, as well as on the metastatic capability of the cells, is reversible upon removal of the drug [74]. These studies support the notion that the expression of vimentin is related to cell shape and/or to the organization of the vimentin network.

The analysis of the IF protein pattern of variants of a pancreatic adenocarcinoma displaying high and low metastatic potentials, reveal that the highly metastatic cells, which grow in culture as adherent but round cells [53], express very low levels of vimentin, but high levels of a characteristic pattern of cytokeratins (Fig. 10 [75]). The flat and well spread nonmetastatic variant expresses, on the other hand, only vimentin and no cytokeratins. In this flat, tumorigenic, but nonmetastatic variant, the expression of vimentin can be reversibly modulated by growing the cells on non adhesive substrates, where the cells attain a round configuration and down-regulate the expression of vimentin, but reexpress vimentin in a few hours after cells attach and spread on the substrate (Fig. 11, [75]).

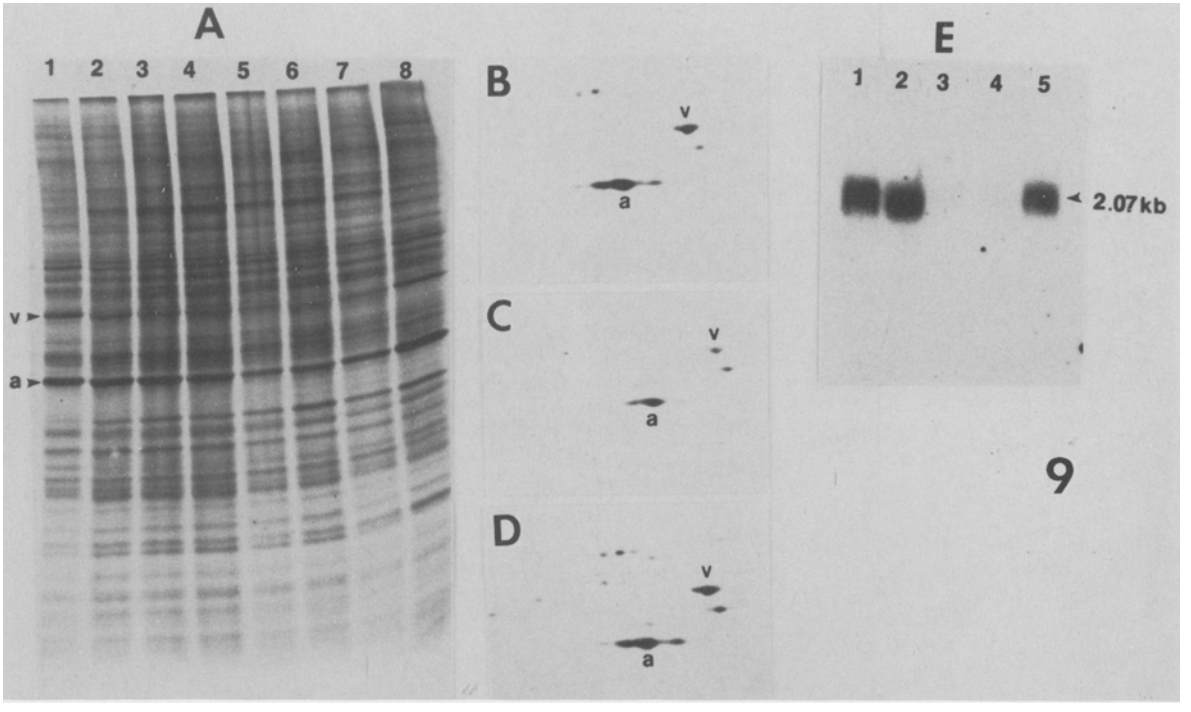

Fig. 9. Reversible decrease in vimentin synthesis and mRNA content following treatment with cycloheximide (CH). B16-F1 cells were treated with CH (A) for 2 h (lane 2), 4 h (lane 3), 6 h (lane 4), 8 h (lane 5), 11 h (lane 6), 16 h (lane 7), or after 16 h of treatment the cells were washed and incubated in fresh medium for 8 h (lane 8). Following these treatments the control (lane 1) and the CH-treated cells were labeled for 1 hr with [35_S] methionine in medium without CH and a Triton X-100 insoluble cytoskeletal fraction was analyzed by SDS polyacrylamide gel electrophoresis or by two dimensional gel electrophoresis (B-D). B, control; C, cells treated for 6 h with CH; D, cells treated for 6 h with CH and then incubated in fresh medium for 16 h. E, poly(A)-containing RNA was isolated from control (lane 1) and from cells treated with CH for 4 h (lane 2), 8 h (lane 3), 11 h (lane 4) and 11 h followed by 8 h recovery in fresh medium (lane 5). The RNA was separated on agarose gels and RNA blots were probed with [^{32}P]-labeled vimentin cDNA. a, actin; v, vimentin; kb, kilobase. (Reprinted with permission from Ben-Ze'ev and Raz [74]).

However, the attachment to the substrate does not appear to influence the synthesis of cytokeratins in the highly metastatic cell variant, since the same cytokeratins are synthesized when the cells are grown in suspension (Fig. 11).

Similar switches in the expression of vimentin and cytokeratins are observed in mesotheliomas [76, 77], in several variants of murine sarcoma 180 cells [78], and in metastatic and non metastatic variants of certain hepatomas [79]. Furthermore, metastatic tumor cells of epithelial origin in ascites or in the pleural fluid express cytokeratins and vimentin, while solid carcinoma expresses only cytokeratins [80]. These studies are in line with our findings with the BSp73 adenocarcinoma variants and suggest that differences in the micro-environment from which the cell lines are derived may confer changes in the expression of intermediate filament proteins in the various cell lines [75].

In several in vitro systems it has been demonstrated that the expression of intermediate filament proteins can be modulated. In many epithelial cells, vimentin synthesis begins soon after the cells are placed in culture [81]. In cells where vimentin is co-expressed with cytokeratins in vivo, its expression is demonstrated in epithelial cells that migrate out from the epithelium, as in the case of the parietal endoderm during embryogenesis [82]. Similarly in bladder epithelial cells treated with a chemical carcinogen, the additional expression of vimentin

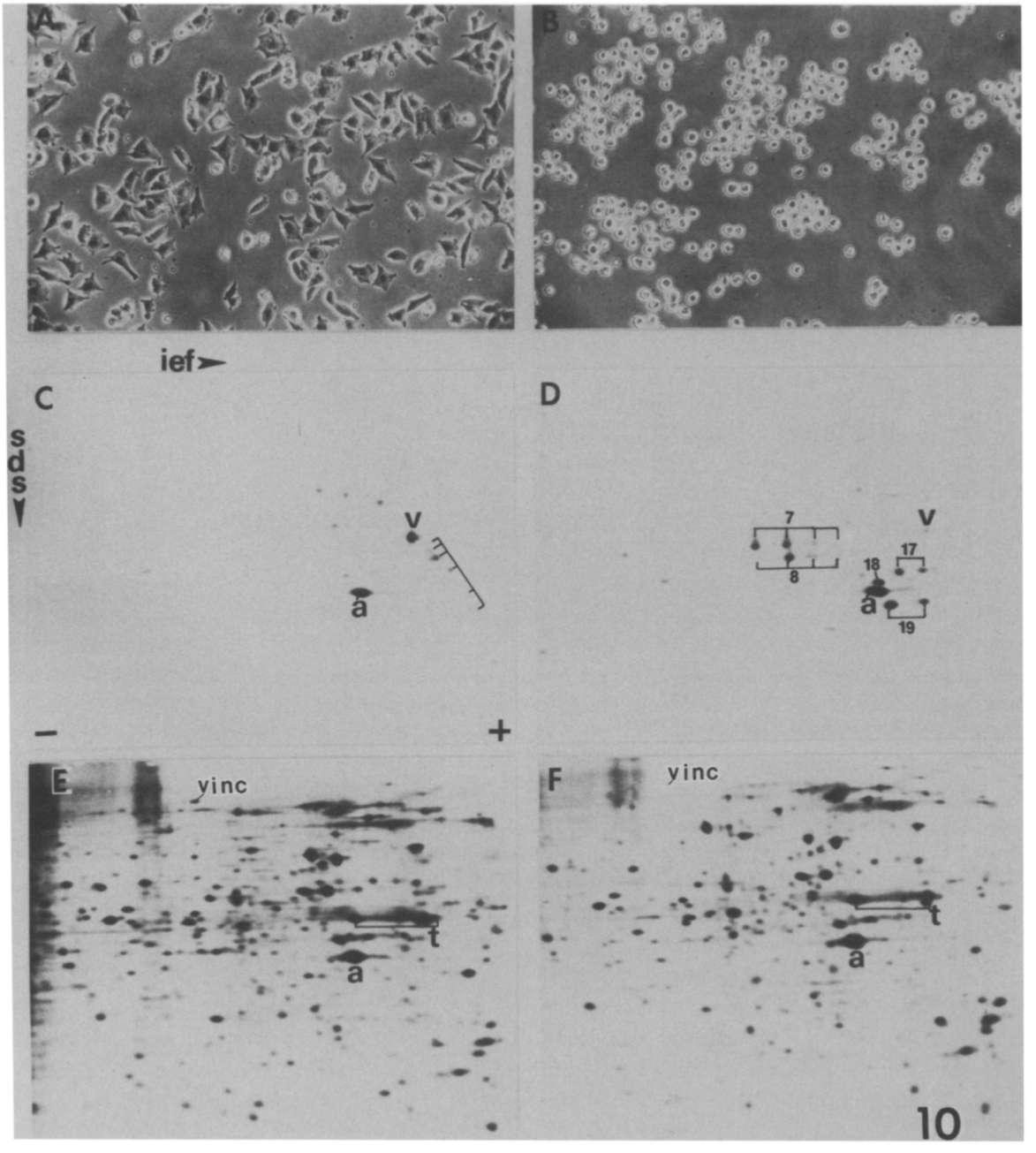

Fig. 10. The morphology and pattern of proteins synthesized by variants of the BSp73 tumor. AS (A, C, E) low metastatic and ASML (B, D, F) high metastatic cells were labeled with [35_S] methionine and the Triton X-100 and high salt soluble fractions (C, D), enriched in intermediate filaments, as well as the Triton-soluble fractions (E, F) were analyzed by two dimensional isoelectric focusing and SDS polyacrylamide gel electrophoresis. a, actin; t, tubulins; v, vimentin; vinc, vinculin; 7, 8, 17, 18, 19, cytokeratins. (Reprinted with permission from Ben-Ze'ev et al. [75]).

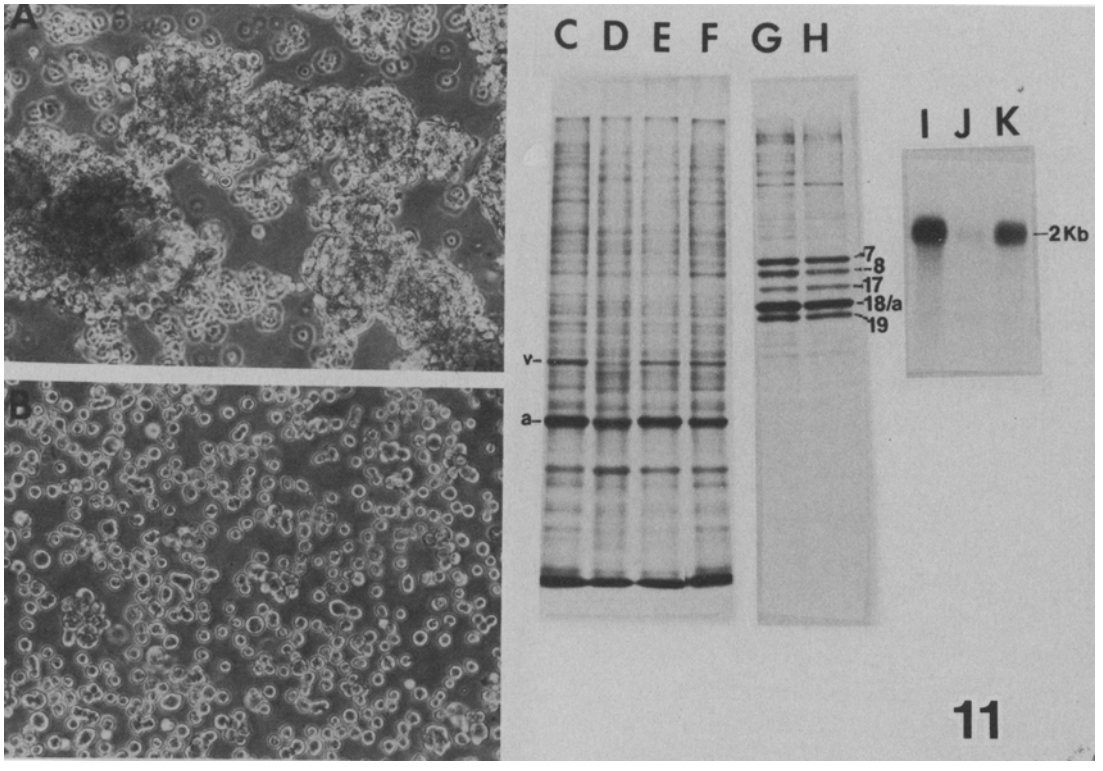

Fig. 11. Modulation of vimentin synthesis in AS cells during suspension culture. AS cells (A, C-F, and I-K) and ASML cells (B, G and H) were grown either on plastic (C, G, and I) or in suspension on poly(HEMA)-coated dishes (A, B, D, H and J). After 3 days the cells were labeled with [35_S] methionine and a Triton X-100 and high salt insoluble fraction was analyzed on 8% (C-F) or 10% (G and H) acrylamide SDS gels. Cells were also replated after 3 days suspension culture for 4 h (E) or 8 h (F) and labeled with [35_S] methionine, or RNA was extracted from cells 4 h after replating (K). Similar amounts of poly(A)-containing RNA from the cytoplasm of monolayer (I), 3 days suspension grown (J), and 4 h replated AS cells (K) were separated on agarose gels and the RNA blots were hybridized with [32_P] DNA from plasmids containing vimentin sequences. kb, kilobase; a, actin; v, vimentin; 7, 8, 17, 18, 19, cytokeratins. (Reprinted with permission from Ben-Ze'ev et al. [75]).

is observed [83]. In established cultures of epithelial cell lines that co-express vimentin and cytokeratins the expression of vimentin was found to be related to the extent of cell spreading on the substrate and similar to cells of mesenchymal and other embryological origins [84]. On the other hand, the synthesis of cytokeratins was maximal with extensive cell-cell contacts that encouraged the formation of cytokeratin-containing desmosomal intercellular junctions and was independent of cell shape changes [72, 84]. Thus, a low level of vimentin expression is obtained in cells where culture conditions impose a spherical configuration on cells as demonstrated in B16 melanoma [51, 72] and as we observed in the spherical variant of the BSp73 ASML cell line [75].

The switches in the synthesis of intermediate filament proteins either by experimental modulation of cell shape and contacts [42, 72], or as displayed by the spontaneous tumor cell variants of the BSp73 system [75] can be related to the metastatic capacity of the various cell types. The availability of both experimental and spontaneous systems might be helpful in further studies aimed at elucidating the relationships among intermediate filament protein expression, cell contact phenomena and the malignant metastatic properties of the various cell lines.

Conclusions

Interactions among cells and of cells with exogenous substrates play a central role in growth control, differentiation and tissue morphogenesis. Changes in cell-shape and -contacts induced by environmental factors can affect such cellular interactions and this may affect gene expression.

The molecular aspects of these interactions of the cell via specific receptors with components of the extracellular matrix on the one hand and with elements of the cytoskeleton on the other hand is under intensive investigation [54]. Elements of these structural cellular assemblies are, most probably, major targets for modification during the conversion of normal cells to malignant cells. The studies presented in this paper have shown that the acquisition of malignant invasiveness by tumor cells may involve the cytoskeletal elements at many levels; during the alterations in cell contacts with other cells and with the extracellular matrix, following the changes in cell shape while in transit via the circulation to distal organs, or during the pathobiological conversions in gene expression and growth control characteristic to these cells. More specifically, in several malignant metastatic tumor systems:

- High metastatic cells display a higher degree of motility than their low metastatic counterparts.
- The metastatic potential of tumor cells can be modulated in a reversible way by culturing the cells in vitro under conditions of varied cell shape.
- The selection of tumor cell clones with altered cell substrate contact properties, in many cases, is followed by the co-selection of altered metastatic properties.
- There are malignant metastatic cell variants that spontaneously express both altered metastatic properties and different cell spreading characteristics on the substrate.
- High metastatic cells of various tumor systems display a lower level of actin organization and vinculin when compared to low metastatic variants which have well developed stress fibers terminating in large vinculin-containing plaques.
- The pattern of intermediate filament expression in certain malignant metastatic cells can be modulated by environmental conditions that affect cell contacts and configuration.

Key unanswered questions

While an association was demonstrated between tumorigenic and metastatic potential and between the organization and expression of various structural cellular assemblies which determine cell contacts and configuration, we are still far away from understanding the molecular basis for these phenomena. Therefore, a major effort in this area is directed toward the investigation of the putative molecules that link elements of the extracellular matrix and the cytoskeleton. In addition, molecules that determine cell adhesion to other cells, and which connect the cytoskeleton to these specialized areas of the membrane, are beginning to be identified. The study, at the molecular level of interaction between these complex structural assemblies in both normal and cancer cells and their relationship to metastasis, will help in our understanding of both tissue morphogenesis and the process of malignant conversion.

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